C3 cont through S225 of the CD43 precursor sequence.--

REMARKS

Applicants have discovered that it is possible to create artificial P-selectin ligands by combining an amino acid sequence containing a tyrosine sulfation site from one polypeptide with an amino acid sequence containing a sialyl Le^x addition site from a different polypeptide, by inserting such sequences into a carrier polypeptide, or by repositioning such tyrosine sulfation and sialyl Le^x addition sites, relative to one another, within the same polypeptide. In general, therefore, applicants' claimed invention features purified nucleic acids encoding artificial P-selectin ligand polypeptides that contain a tyrosine sulfation site and a sialyl Le^x addition site, wherein at least one of the sites is located at an amino acid position at which it does not naturally occur. The invention also features vectors and cells containing the claimed nucleic acids.

Summary of the Office Action

Claims 10 and 12-14 stand rejected under 35 U.S.C. §§ 103 and 112, first and second paragraphs. In addition, claims 1-9, 11, and 15-23 are withdrawn from consideration as being drawn to a non-elected invention and species; applicants are informed that the submitted drawings and photographs fail to comply with 37 C.F.R. § 1.84; and applicants are reminded to amend the Brief Description of the Drawings in

accordance with form PTO-948.

Support for the Amendments

Claim 10 has been amended to clarify the claim language. Support for the amendment to claim 10 may be found, e.g., at page 16, lines 5-14, and at page 24, lines 20-24. In addition, new claims 24 and 25, which depend from claim 10, have been added. Support for claim 24 may be found, e.g., at page 16, lines 5-15 of the specification, and support for claim 25 may be found, e.g., at page 12, lines 14-17 of the specification, as well as at page 16, lines 5-15, at page 18, lines 15-16, and at Fig. 14. No new matter has been added by these amendments.

Drawings

Applicants note the objection to the drawings, and, as stated in the previous Reply, will provide formal drawings when otherwise allowable subject matter has been indicated.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 10 and 12-14 stand rejected under 35 U.S.C. § 112, first paragraph. The Office Action asserts that the specification does not contain a written description of the claimed invention, and that the claimed invention is not enabled. These grounds for

rejection are addressed in sections (A) and (B) below.

(A) Rejection based on the written description requirement

The Office Action asserts that the specification does not support the use of the word "artificial" in claim 10. In response, applicants have replaced "artificial" with "synthetic." Support for the term "synthetic P-selectin ligand" is found throughout the specification (e.g., at page 10, lines 13-14, at page 16, lines 5-6, and at page 24, lines 1-2), and this aspect of the rejection may now be withdrawn.

The Office Action also asserts that the broad recitation of sialylation and sulfation "consensus sequences" in claim 10 changes the scope of the claims and is not supported by the disclosure of sites and repeat sequences derived from the sequences of Factor VIII, the fourth component of complement, PSGL-1, and CD43. In response, applicants have amended claim 10 such that it now recites a nucleic acid encoding a polypeptide that contains a "sialyl Le^x addition site" and a "tyrosine sulfation site." These terms are clearly supported by the specification. For example, the term "sialyl Le^x addition site" (sometimes referred to as a "glycan addition site") is found throughout the specification, e.g., at page 9, lines 11-12, and at page 24, lines 20-22. Similarly, the term "tyrosine sulfation site" is found at several places within the specification, e.g., at page 22, line 10.

As amended, claim 10 clearly meets the written description requirement set forth under 35 U.S.C. § 112, first paragraph. According to the MPEP § 2163.02 (Rev. 3, July

1997)

> An objective standard for determining compliance with the written description requirement is, "does the description clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed [emphasis added]."

In agreement with this standard, the specification, in combination with what was already known, would have allowed a person of ordinary skill in the art to readily recognize that applicants had invented the nucleic acids as currently claimed.

For example, at page 3, lines 4-8, the specification states that the invention features "nucleic acid encoding a protein containing sites for the attachment of a sialyl Le^x determinant and a sulfated determinant, at least one of these determinants being positioned at a non-naturally occurring site on the protein" and "nucleic acid encoding any one of the P-selectin ligands of the invention"; on page 5, lines 19 through 20, "P-selectin ligand" is defined as "any amino acid sequence capable of mediating an interaction with the P-selectin receptor." Specific examples of such ligands are described at page 24, lines 1 through 16, and explanations for determining whether a polypeptide is a P-selectin ligand are provided within the specification, e.g., at page 13, line 11, through page 14, line 3. On page 29, lines 6 through 12, the specification states that

> to create a molecule that blocks P-selectin-mediated interactions [i.e., a molecule that itself interacts with the P-

> selectin receptor] sites for sulfation and if necessary, sialyl-Le^x addition may be introduced into an antibody fusion protein...Such sites may be incorporated...for example, by introducing one or more sulfation sites (i.e., a tyrosine in an acidic context) in the vicinity of an introduced or existing sialyl-Le^x addition site (for example, by standard techniques of site-directed mutagenesis)... [emphases added]

These and other statements contained within the specification clearly indicate to one of ordinary skill in the art that applicants had indeed invented the scope and content of the nucleic acids presently claimed. In particular, applicants submit that, even though the claimed invention is exemplified by the specific sialyl Le^x and tyrosine sulfation sites described in the present specification, one of skill in the art reading this specification would have readily recognized that these sites were merely provided for the purpose of illustrating the invention and that applicants' invention included the use of any convenient tyrosine sulfation or sialyl Le^x addition site for synthetic P-selectin ligand production.

With respect to the claim terms themselves, applicants submit that the name and characteristic features of each of these sites is well understood by skilled practitioners in this area of technology. As evidence of this assertion, the Examiner is directed to the publications submitted with this Reply. Hansen et al. (*Biochem. J.* 308:801-813, 1995), for example, describes a computer server for predication of O-linked glycosylation sites (see end of abstract), which had been publicly available before the filing date of this application. Schmid et al. (*Proc. Natl. Acad. Sci. USA* 89:663-667, 1992) discloses the

amino acid sequence for the extracellular domain of CD43/sialophorin/leukosialin, and specifically points out the sites for sialyl Le^x attachment (i.e., the N-glycan and O-glycan addition sites) within the amino acid sequence (see, e.g., Fig. 1). And Bause (*Biochem. J.* 209:331-336, 1983, abstract attached), Roitsch and Lehle (*Eur. J. Biochem.* 181:525-529, 1989, abstract attached), and Beeley (*Biochem. J.* 159:335-345, 1976, abstract attached) teach amino acid sequence motifs that promote N-linked glycosylation, as well as motifs that inhibit glycosylation. In sum, the description in the specification of a "nucleic acid encoding a protein that contains a sialyl Le^x addition site" would have been well understood by a skilled artisan, and thus satisfies the written description requirement.

Likewise, the claim term "nucleic acid encoding a protein that contains a tyrosine sulfation site" would also have been readily understood by any skilled artisan in this area, as the sequence requirements for tyrosine sulfation sites were also well-defined. As evidence of this assertion, applicants submit the attached publications by Hortin et al. (*Biochem. and Biophys. Res. Comm.* 141:326-333, 1986, e.g., see page 331, paragraph 3) and Huttner (*Ann. Rev. Physiol.* 50:363-376, 1988, e.g., see page 369, Table 2), both of which extensively discuss the amino acid consensus requirements for tyrosine sulfation. The Examiner's attention is also drawn to Rosenquist and Nicholas (*Protein Science* 2:215-222, 1993), a paper which describes a computer-based analytical approach that allows prediction of tyosine sulfation sites with almost 100% accuracy. Moreover, the attached publication by Niehrs et al. (*J. Biol. Chem.* 267:15938-15942, 1992, abstract

attached) describes the construction of an artificial gene encoding a polypeptide that contains twelve repeats of a heptaheptide amino acid motif corresponding to a previously-identified tyrosine substrate motif, and shows that each of the twelve tyrosyl residues within the artificial protein was stoichiometrically sulfated within transfected cells, indicating that the heptaheptide motif was sufficient to direct tyrosine sulfation. In view of these references, as well as the numerous other references available as of the date of the present application, applicants submit that a skilled artisan would have understood what was meant by "tyrosine sulfation site" as stated in the present specification and as now recited in the present claims.

In sum, present claims 10 and 12-14 comply with the written description requirement set forth in 35 U.S.C. § 112, first paragraph, and this aspect of the rejection may now be withdrawn.

(B) Rejection based on the enablement requirement

The Office Action also asserts that the specification provides insufficient guidance to assist a skilled artisan in the selection of "artificial ligands" and "consensus sequences" and that the specification fails to provide any general guidance for making and using useful nucleic acids encoding a "myriad of artificial ligands and consensus sequences."

Applicants submit that the current claims are clearly enabled. As stated in the previous Reply (filed October 15, 1998), the teachings of the specification, combined

with what was already known in the art, would have been sufficient to allow a skilled artisan to make and use the claimed nucleic acids; the present Office Action does not dispute this fact. For instance, one exemplary nucleic acid, described at page 24, lines 1-16 of the specification, encodes the Factor VIII tyrosine sulfation sequence fused upstream from a nucleic acid encoding a CD43 fragment consisting of Ile135 through the carboxy terminus of CD43 (encompassing the membrane proximal, transmembrane, and intracellular domains of CD43), which, as previously known in the art, contains sialyl Le^x addition sites. This nucleic acid construct encodes an artificial polypeptide, in which the CD43 glycosylation sequences of the proximal domain are fused to a sulfation consensus sequence located at an amino acid position within the CD43 polypeptide at which this sulfation sequence does not naturally occur. This polypeptide functions as an artificial P-selectin ligand.

As discussed above, the amino acid sequence requirements for tyrosine sulfation or sialyl Le^x addition have long been known. Similarly, genetic engineering approaches for introducing a given amino acid sequence into a region of a protein have long been known. And determination of whether an engineered polypeptide behaves as an artificial P-selectin ligand may be readily achieved by routine screening methods that are described within the specification. Since "enablement is not precluded by the necessity for some experimentation such as routine screening" (*In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988)), the need for such routine experimentation to identify particularly

favorable embodiments of the claimed nucleic acids is not sufficient to support a rejection of the claims for lack of enablement.

In response to the assertion that it would take undue experimentation to produce all possible artificial P-selectin ligands and consensus sequences covered by the claims, applicants point out that nowhere does the patent statute or the case law require that the specification of a patent application teach how to make or how to use all possible species covered by a claim, and that applicants' specification clearly facilitates the production and use of a reasonable number of synthetic P-selectin ligands.

In sum, any person having ordinary skill in the art, using the teachings of the specification combined with what was already known in the art, would readily have been able to make and use the claimed nucleic acids. Accordingly, claims 10 and 12-14 are enabled, under 35 U.S.C. § 112, first paragraph, and this rejection may be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 10 and 12-14 also stand rejected under 35 U.S.C. § 112, second paragraph. Specifically, the Office Action asserts that the characteristics of the recited "artificial P-selectin ligand" and the "consensus sequences" are ambiguous and unclear. Applicants have amended the claims to recite the terms "synthetic P-selectin ligand," "sialyl Le^x addition site," and "tyrosine sulfation site." As explained above, these terms are perfectly clear and unambiguous in view of the specification and what was known in the art.

According to the § 2173.05(a) of the MPEP (Rev. 3, July 1997) "The meaning of every term used in a claim should be apparent from the prior art or from the specification and drawings at the time the application is filed." This standard is clearly met by the amended claims, in view of the present specification and what was already known in the art; therefore, this aspect of the rejection may be withdrawn.

The Office Action also asserts that there is insufficient biochemical (e.g., sequence) information that distinctly identifies the "P-selectin ligand" and "consensus sequences" encompassed by the claimed invention, and that the recitation of "artificial ligands" and "consensus sequences" fails to distinctly claim what the polypeptide is and what the compositions are made up of. The Office Action also asserts that the claim language is vague and indefinite since it "encompasses a myriad of different artificial ligands and consensus sequences." In response, applicants point out that, according to the MPEP § 2173.04 (Rev. 3, July 1997)

> Breadth of a claim is not to be equated with indefiniteness. *In re Miller*, 441 F.2d 689, 169 USPQ 597 (CCPA 1971). If the scope of the subject matter embraced by the claims is clear...then the claims comply with 35 U.S.C. 112, second paragraph.

The scope of the subject matter embraced by claim 10, as amended, is clear and definite: the claim reads on any nucleic acid encoding a polypeptide that behaves as a P-selectin

ligand and contains a sialyl Le^x addition site and a tyrosine sulfation site, at least one of which is at a position within a protein at which it does not naturally occur (i.e., it is artificially introduced into the protein). The specification in combination with the prior art clearly teaches the characteristics of a P-selectin ligand and the amino acid compositions of sialyl Le^x addition sites and tyrosine sulfation sites. Given this description, one of ordinary skill in the art would clearly understand the scope and content of the claims as now amended. The § 112, second paragraph rejection may be withdrawn.

Rejections under 35 U.S.C. § 103

Claims 10 and 12-14 stand rejected, under 35 U.S.C. § 103, for obviousness over Larsen et al. (U.S. Patent No. 5.843,707; "Larsen"), Sasaki et al. (*J. Biol. Chem.* 269:14730, 1994; "Sasaki"), or Sako et al. (*Cell* 75:1179-1186, 1993; "Sako"), in view of Aruffo et al. (*Cell* 67:35-44, 1991; "Aruffo") and Lowe (U.S. Patent No. 5,595,900).

The claims, as amended, recite a nucleic acid encoding a polypeptide that is a synthetic P-selectin ligand and that contains a sialyl Le^x addition site and a tyrosine sulfation site, wherein at least one of these sites is located at an amino acid position that is different from its position in a naturally occurring P-selectin ligand. Because none of the cited reference combinations teaches or suggests the invention as now claimed, this rejection is respectfully traversed.

In particular, Larsen teaches P-selectin ligands fused to carrier molecules (such as immunoglobulins); however, these fusion proteins contain only naturally occurring sites for sulfation and glycosylation. By contrast, the amended claims recite a nucleic acid encoding a synthetic P-selectin ligand that has at least one sulfation or glycosylation site at a non-naturally occurring position within the polypeptide. The combination of Larsen with Aruffo and Lowe does not cure the deficiency of Larsen, as the references, when combined, still do not teach or suggest the claimed invention.

The Office Action asserts that Aruffo teaches "the importance of sulfation sites for P-selectin binding by suppressing P-selectin binding via the sulfation suppression," and, therefore, it was known at the time the invention was made that both sialyl and sulfation sites contribute to P-selectin binding. However, the Office Action attributes to Aruffo teachings not in the reference.

Aruffo only teaches that sulfatides, which are lipids (specifically, heterogeneous 3-sulfated galactosyl ceramides), are ligands of CD62/P-selectin. Aruffo does not teach any polypeptide P-selectin ligands at all, and certainly does not teach that both sialylation and tyrosine sulfation of a polypeptide P-selectin ligand contribute to its ability to bind to P-selectin.

With respect to Lowe, the Office Action states that this reference teaches "providing nucleic acids that encode for glycosylation and sulfation sites in glycoproteins of interest," specifically citing column 14, paragraph 2 of the reference. This, however, is

a misinterpretation of Lowe. The cited paragraph discusses a method for isolating nucleic acids (genes, cDNAs, and mRNAs) that encode enzymes that post-translationally modify protein substrates by glycosylating, sulfating, phosphorylating, methylating, acylating, or de-glycosylating the proteins. The cited paragraph does not disclose any nucleic acid encoding an artificial P-selectin ligand that contains sites for both glycosylation and sulfation, nor is such a nucleic acid taught anywhere within the Lowe reference.

Turning to Sasaki, the Office Action states that this reference teaches "selectin ligands that have been modified to express carbohydrate moieties, as well as the nucleic acid, vector, and cells that encode and express said modified ligands." Applicants respectfully disagree with this reading of the Sasaki reference. Sasaki teaches only the cloning of a nucleic acid encoding a novel isoform of a fucosyltransferase, Fuc-TVII, and shows that expression of Fuc-TVII in human Burkitt lymphoma cells increases the binding of the cells to E-selectin. Sasaki, even when combined with Aruffo and Lowe, does not teach or suggest purified nucleic acids that encode artificial P-selectin ligands, nor does Sasaki teach vectors or host cells containing such purified nucleic acids.

Finally, with respect to Sako, the Office Action states that this reference teaches a nucleic acid encoding a P-selectin ligand, as well as the importance of carbohydrate sites in selectin-mediated binding and structure. However, applicants note that Sako does not teach that sulfated tyrosine is necessary for the binding of a P-selectin ligand to P-selectin. Nor does Sako teach the construction of artificial P-selectin ligands as recited in

the present claims. And combining Sako with Aruffo and Lowe does not cure this deficiency.

To summarize, the combination of Larsen, Sasaki, or Sako with Aruffo and Lowe does not teach or suggest the invention as currently claimed. Accordingly, claims 10 and 12-14 cannot be obvious over the cited references, and the § 103 rejection may be withdrawn.

Conclusion

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested.

Enclosed is a petition to extend the period for replying for two months, to and including June 1, 1999, May 29, 30, and 31 being a Saturday, Sunday, and holiday, respectively. If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 1 June 1999

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